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Degradation, Foraging, and Depletion of Mucus Sialoglycans by the Vagina-adapted *Actinobacterium Gardnerella vaginalis*[*] [S]

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Background: Mucus degradation is hypothesized to be important in bacterial vaginosis (BV), but mechanisms require investigation.
Results: We characterize a *Gardnerella vaginalis* pathway that performs digestion/catabolism of mucus sialoglycans.
Conclusion: *G. vaginalis* participates in mucosal sialic acid depletion in BV.
Significance: *G. vaginalis* is the first example of a BV-associated organism that recapitulates a measurable clinical phenotype of mucus degradation in an animal model.

Bacterial vaginosis (BV) is a polymicrobial imbalance of the vaginal microbiota associated with reproductive infections, preterm birth, and other adverse health outcomes. Sialidase activity in vaginal fluids is diagnostic of BV and sialic acid-rich components of mucus have protective and immunological roles. However, whereas mucus degradation is believed to be important in the etiology and complications associated with BV, the role(s) of sialidases and the participation of individual bacterial species in the degradation of mucus barriers in BV have not been investigated. Here we demonstrate that the BV-associated bacterium *Gardnerella vaginalis* uses sialidase to break down and deplete sialic acid-containing mucus components in the vagina. Biochemical evidence using purified sialoglycan substrates supports a model in which 1) *G. vaginalis* extracellular sialidase hydrolyzes mucosal sialoglycans, 2) liberated sialic acid (*N*-acetylneuraminic acid) is transported into the bacterium, a process inhibited by excess *N*-glycolylneuraminic acid, and 3) sialic acid catabolism is initiated by an intracellular aldolase/lyase mechanism. *G. vaginalis* engaged in sialoglycan foraging *in vitro*, in the presence of human vaginal mucus, and *in vivo*, in a murine vaginal model, in each case leading to depletion of sialic acids. Comparison of sialic acid levels in human vaginal specimens also demonstrated significant depletion of mucus sialic acids in women with BV compared with women with a "normal" lactobacilli-dominated microbiota. Taken together, these studies show that *G. vaginalis* utilizes sialidase to support the degradation, foraging, and depletion of protective host mucus barriers, and that this process of mucus barrier degradation and depletion also occurs in the clinical setting of BV.

One in three women have bacterial vaginosis (BV)[3] at any point in time (1), a microbial imbalance of the female reproductive tract characterized by a lack of healthy bacteria in the vagina (mainly lactobacilli). Instead, the vaginal flora in BV consists of a complex community dominated by Gram-negative anaerobes, *Actinobacteria,* and other bacteria. Women with BV are at increased risk of pelvic inflammatory disease, postsurgical infections, sexually transmitted infections, and serious pregnancy complications such as intrauterine infection and preterm birth (2–17). Recent advances in DNA sequencing technologies and other molecular tools have provided an unprecedented view of the diversity and longitudinal variability of the vaginal bacterial microbiota (18–21). Such modern microbiome studies confirm and extend previous culture-based approaches, further deepening our view of the polymicrobial nature of BV (7, 22, 23). BV is common, and often asymptomatic from the patient perspective, but displays characteristic clinical features (also known as Amsel criteria) including increased pH, thinning of vaginal fluid secretions (or "abnormal discharge"), a fishy odor upon potassium hydroxide treatment of vaginal specimens, and the presence of "clue" cells (epithelial cells studded with bacteria) in wet mounts (24, 25). Recent work has demonstrated some correlations between clinical features of BV and the presence of particular BV-associated bacteria (26). However, experimental models are required to examine how individual bacterial species or strains interact with the host.

Glycosylated proteins that display outermost sialic acid residues (sialoglycoproteins) are major constituents of mucus and participate in the exclusion of potential pathogens from mucosal surfaces (27). Mucins are heavily glycosylated mucus proteins, consisting of up to 16% sialic acids by weight, and they are thought to provide a thick lubricated physical barrier that prevents close contact of pathogens with epithelial cells (28, 29). Immunoglobulins are another example of secreted sialoglycoproteins with mucosal immune functions (30, 31). A number of

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[3] The abbreviations used are: BV, bacterial vaginosis; DMB, 1,2-diamino-4,5-methylenedioxybenzene; BSM, bovine submaxillary mucin; Neu5Ac, *N*-acetylneuraminic acid; Neu5Gc, 5-*N*-glycolylneuraminic acid.

studies suggest that degradation of mucus components may be a key factor in the etiology of BV and BV-associated adverse health outcomes. BV has been associated with the presence of multiple hydrolytic activities in vaginal fluids, especially glycosidases (32–34). In fact, sialidase enzyme activity in vaginal fluids is now used as a diagnostic indicator of BV (14, 35–38) and has been independently correlated with risk of ascending infections and preterm birth (6, 39–41). However, whereas current evidence strongly suggests that BV-associated bacteria produce hydrolytic enzymes that degrade protective mucus barriers, the mechanisms of mucus degradation employed by individual species or communities of bacteria in BV have not been characterized.

Gardnerella vaginalis is the most frequently isolated bacterium associated with BV, and produces a sialidase hypothesized to participate in the degradation of mucus (32, 34, 42, 43). However, relatively little is known at the molecular level about the relationship between *G. vaginalis* and its human host (44, 45). *G. vaginalis* was the first bacterium isolated from women with BV, although at that time the condition was referred to as "nonspecific vaginitis," and *G. vaginalis* was mistakenly identified as a Gram-negative bacterium "*Haemophilus vaginalis*" (46). More than half a century later, we know that this organism is actually a member of the phylum *Actinobacteria* (also known as the high-GC Gram-positives). Studies showed that recovery of *G. vaginalis* from vaginal fluids was 92% sensitive and 69% specific in identifying women with BV, as diagnosed by Amsel criteria (3 of 4 subjective measures) (23). Many other studies have reproduced this strong correlation between overgrowth of *G. vaginalis* and BV. However, the potential role of *G. vaginalis* in the etiology of BV remains controversial because women with apparently "normal" microbiota at the time of sampling can also be carriers of *G. vaginalis* (23, 47). Consistent with the role of *G. vaginalis* as a potential pathogen, culture-based studies recovered the bacterium from placentas of 26% of women delivering preterm with histological evidence of chorioamnionitis (9). *In vitro* studies have further described the pathogenic potential of *G. vaginalis* in cell adhesion and entry, cytolytic toxin production, and biofilm formation (2, 48, 49) and computational studies revealed that the presence of *G. vaginalis* is strongly correlated with clinical phenotypes of BV (26). Taken together, these studies support the hypothesis that *G. vaginalis* is an active participant as opposed to an innocent bystander in BV. However, further experimental study is required to demonstrate active participation of *G. vaginalis* in phenotypes associated with BV.

Here we present biochemical, cellular, and *in vivo* investigations of *G. vaginalis,* demonstrating that the bacterium can engage in the active degradation and foraging of mucus substrates, using sialidase to release sialic acid residues from mucus sialoglycoproteins, and subsequently capturing and catabolizing the free monosaccharide. We show that the hydrolysis and metabolism of sialic acid residues by *G. vaginalis* occurs in both *in vitro* and *in vivo* models and that *G. vaginalis* is sufficient to induce a sialoglycan-depleted state in a murine vaginal infection model. These experiments provide the first evidence of an individual BV-associated bacterium that participates in mucus degradation, a process believed to underlie the increased susceptibility to ascending uterine infections in women with BV. These studies also demonstrate that *G. vaginalis*, a species of previously controversial significance in BV, is in fact sufficient to hydrolyze mucus sialoglycans and deplete the vaginal mucosa of sialic acids during infection. Additional evidence strongly suggests that this process of sialoglycan foraging also leads to sialic acid depletion in the clinical condition of BV, further underscoring the potential clinical relevance of these findings.

EXPERIMENTAL PROCEDURES

Isolation of G. vaginalis Strains from Clinical Specimens—Vaginal swabs were obtained from women enrolled in the Washington University Contraceptive CHOICE Project (IRB ID 201108155) and transported from the clinic to the lab using Port-A-Cul™ pre-reduced anaerobic transport media tubes (BD Bioscience). Within 24 h, tubes were brought into a vinyl anaerobic airlock chamber (Coy Laboratory Products) under an atmosphere maintained at ~1% hydrogen and 0 ppm oxygen, and swabs were used to inoculate "Gardnerella semiselective media" (Columbia agar plates with 5% defibrinated sheep blood, 10 mg/liter of colistin, 10 mg/liter of nalidixic acid, and 4 mg/liter of amphotericin B). Plates were preincubated in the chamber for at least 16 h for equilibration to anaerobic conditions and were incubated anaerobically postinoculation at 37 °C for 24–48 h, followed by inspection for translucent pinpoint colonies. Candidate isolates were streaked to isolation followed by diagnostic PCR using forward primer GGGCGGGGCTAGAGTGCA and reverse primer GAACCCGTGGAATGGGCC reported to be selective for *G. vaginalis* (50). Additional validation of *G. vaginalis* strain identity was obtained by sequencing 16 S rDNA (GenBank™ accession numbers have been provided in Table 1).

Culture, Storage, and Recovery of G. vaginalis—For liquid culture, *G. vaginalis* clinical isolates and the reference strain ATCC14019 were cultured in ATCC NYC-III media containing horse serum. For glycerol freezer stocks, cultures grown for 28–48 h were supplemented with 3 volumes of *G. vaginalis* freezing additive (autoclaved 6.7% glycerol, 1.3% protease peptone) and stored at −80 °C (51). To recover from −80 °C glycerol stocks, bacteria were streaked to isolation on *Gardnerella* semiselective media as described above under anaerobic conditions in a vinyl anaerobic chamber (Coy). For experiments analyzing sialic acid consumption during *G. vaginalis* growth in NYC-III media, 24–48-h starter cultures were diluted into fresh media to an A_{600} (optical density at 600 nm) of 1.0, and 1200 μl of this normalized culture was centrifuged at 10,000 × *g* for 10 min followed by removal of the supernatant under anaerobic conditions and resuspension of the pellet in 2 ml of fresh media. 100 μl of this bacterial suspension was used to inoculate 4-ml cultures, followed by evaluation of sialic acid content at the indicated time points as described below.

Sialidase Activity Assays—G. vaginalis isolates were grown anaerobically in NYC-III media overnight at 37 °C and A_{600} was measured in a spectrophotometer. Whole cultures or culture supernatants were then diluted 20-fold into 100 mM sodium acetate, pH 5.5, containing 460 μM 4-methylumbelliferyl-Neu5Ac. Substrate hydrolysis was monitored using the fluores-

cence of 4-methylumbelliferyl in a Tecan M200 plate reader every 2 min as previously described (52). Relative sialidase activities/s were normalized to bacterial density.

Measurement of Total and Free Sialic Acids by DMB-HPLC—Derivatization and quantitation of sialic acids by HPLC was carried out as previously described (27, 52–57)(see supplemental Fig. S1 for an example raw data of bovine submaxillary mucin sialic acids and *N*-acetylneuraminic acid standards). For measurement of total sialic acids (including bound and free), mild acetic acid hydrolysis of samples or specimens (2 N acetic acid for 3 h at 80 °C) was performed prior to derivatization with DMB (1,2-diamino-4,5-methylenedioxybenzene) as described below. Alternatively, free sialic acid levels present in samples or specimens were measured by DMB derivatization without prior acid hydrolysis. Thus, the concentration of bound sialic acids is measured indirectly (bound = total − free). All samples and specimens were filtered prior to derivatization over a 10,000 molecular weight cutoff centrifugal filtration device (Vivaspin). Reaction conditions for DMB derivatization were 7 mM DMB, 22 mM sodium thiosulfite, 0.75 M 2-mercaptoethanol, and 1.4 M acetic acid for 2 h at 50 °C. Derivatized samples were injected into a Waters HPLC equipped with a reverse-phase C18 column (Tosoh Bioscience) and eluted using isocratic conditions at 0.9 ml/min using 8% methanol, 7% acetonitrile in water. An on-line fluorescence detector (Waters) was set to excite at 373 nm and detect emission at 448 nm. Peak integrations were used to quantitate sialic acid content by referencing a standard curve of pure sialic acid (Neu5Ac, Sigma) derivatized in parallel. Unless otherwise noted in experiments using Neu5Gc, quantitation of "sialic acid" refers to Neu5Ac.

Biochemical Analysis of G. vaginalis Hydrolysis and Utilization of Sialoglycan Substrates—G. vaginalis strains were grown anaerobically overnight in 5 ml of NYC-III medium at 37 °C. NYC-III contains horse serum. Neu5Gc is a minor contributor to total sialic acids in NYC-III (about 10%). Bacteria were pelleted and washed in 1 ml of 100 mM sodium acetate, pH 5.5. The cells were then resuspended and diluted in acetate buffer to give an A_{600} of 3.2. The bacterial suspensions were supplemented with human myeloma serum immunoglobulin A-IgA (Kent Laboratories) or bovine submaxillary mucin-BSM (Sigma) at a final concentration of 400 μg/ml. This mixture was then dispensed in 50-μl aliquots to each of 10 tubes and incubated at 37 °C. At each time point, aliquots were centrifuged and 35 μl of supernatant was removed for subsequent DMB derivatization. Samples from each experiment were derivatized in parallel to assess free and total sialic acid content.

Analysis of Free Sialic Acid Uptake and Utilization—G. vaginalis strains were grown overnight, washed, and diluted to OD 3.2 in 100 mM sodium acetate, pH 5.5. Free Neu5Ac was added to 10 μM, and 50-μl aliquots were distributed to fresh tubes for incubation at 37 °C. At each time point, bacteria were pelleted and 35 μl of supernatant was collected for DMB derivatization and HPLC.

G. vaginalis Sialic Acid Aldolase/Lyase Activity Assays—G. vaginalis strains were grown anaerobically overnight in 8 ml of NYC-III broth and washed once in 1 ml of 100 mM sodium acetate, pH 5.5. Following resuspension in 450 μl of sodium acetate buffer, the bacteria were sonicated six times for 10 s in a Sonic Dismembrator (Fisher Scientific) at 20% amplitude using a Microtip with 15-s cooling intervals on ice. Intact cells were pelleted by centrifugation and the clarified supernatant was transferred to a fresh tube. This process was repeated twice more to remove any remaining debris. To test for lyase activity, lysates were supplemented with 100–200 μM Neu5Ac and incubated at 37 °C in 40-μl aliquots. At each time point, an aliquot was removed, diluted 10-fold into 100 mM sodium acetate, pH 5.5, and stored at −20 °C prior to HPLC. To test for aldolase activity, lysates were mixed with pyruvate and *N*-acetylmannosamine, both at 1 mM. Aliquots were incubated at 37 °C and removed at each time point for sialic acid quantification by HPLC.

Biochemical Analysis of G. vaginalis Hydrolysis and Utilization of Sialoglycan Substrates in Human and Mouse Vaginal Mucus—To monitor vaginal mucus sialic acid utilization by *G. vaginalis*, reactions were set up essentially as described above for IgA and BSM. For these assays, eluted human specimens with Nugent scores of 0–3 were validated as having no detectable level of sialidase activity (to eliminate the rare possibility low sialidase activity in the absence of Nugent-defined BV). For mouse vaginal mucus, material was obtained by washing vaginas of uninfected mice with 50 μl of phosphate-buffered saline (PBS) and confirming the absence of sialidase activity as for human samples.

Murine Model of G. vaginalis Vaginal Infection—Animal infection studies were performed in accordance with approved protocols from the Washington University Division of Comparative Research. Female C57/Bl6 mice (6–8 weeks) were injected intraperitoneally with 0.5 mg of β-estradiol in 100 μl of filter-sterilized sesame oil 3 days prior to, and on the day of, inoculation. Mice were anesthetized with isofluorane and inoculated vaginally with $\sim 5 \times 10^7$ colony forming units of *G. vaginalis* in 20 μl of sterile PBS ($A_{600} = 5.0$). Vaginal washes were collected by flushing the vaginas of anesthetized mice with 50 μl of sterile PBS, pipetting up and down 10 times at the vaginal orifice, followed by rinsing into an additional 10 μl of PBS in a sterile 1.5-ml tube using a P200 pipette. Vaginal washes were then analyzed for the presence of free and total sialic acid levels as described above. Recovery of *G. vaginalis* in vaginal washes was confirmed by anaerobic culture on *Gardnerella* semiselective media, followed by colony PCR using *G. vaginalis*-specific primers as described above.

Clinical Specimen Handling and Analysis—Vaginal swabs (Starplex) were collected as part of the Contraceptive CHOICE project (58) according to protocols approved by the Washington University Institutional Review Board (IRB ID 201108155) and stored at −80 °C until use. Specimens underwent Nugent scoring using published methods as previously described (52, 59). Swabs were thawed on ice and eluted in 400–1000 μl of 100 mM sodium acetate buffer, pH 5, in deep 96-well plates in a biosafety cabinet at room temperature. Early experiments (including total sialic acid analyses in vaginal specimens) employed 400-μl elutions, whereas later experiments, including free sialic acid analyses, employed 1000-μl elutions. In general, we found that the larger elution volume increased the total recoverable biological material from the swabs. Elution was allowed to proceed for ~40 min with gentle agitation. Eluted

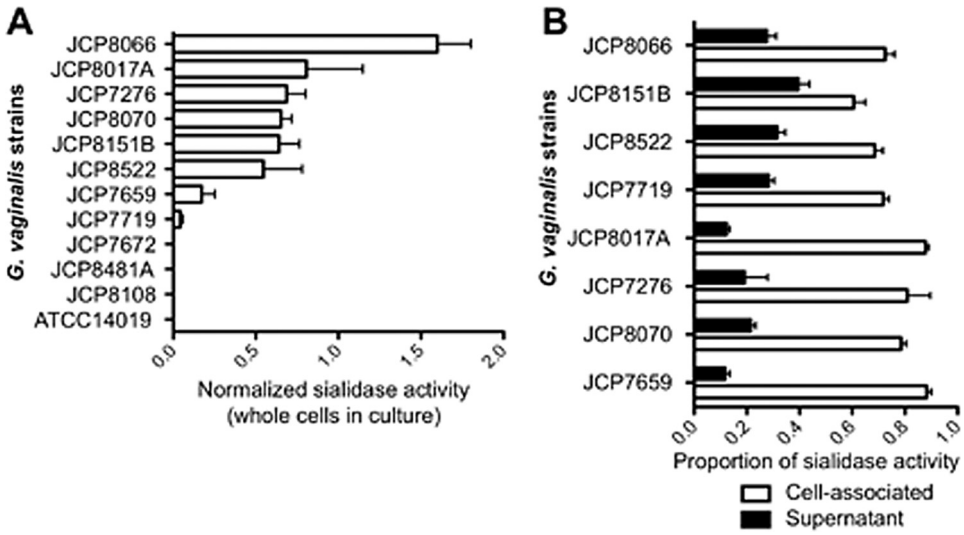

FIGURE 1. ***G. vaginalis* clinical isolates produce cell-associated and secreted sialidase.** *A*, *G. vaginalis* isolates were grown anaerobically in NYC-III media and samples were analyzed for sialidase activity, normalized to the optical density of cultures as described under "Experimental Procedures." *B,* the proportion of cell-associated *versus* secreted sialidase activity was determined by pelleting bacteria and comparing activity remaining in the supernatant with that of the whole culture suspension. *Error bars* represent S.D.

vaginal fluid was used directly in derivatization reactions for analysis of free sialic acid or hydrolyzed with mild acetic acid for analysis of total sialic acid as described below.

Sequence Accession Numbers—See Table 1 for accession numbers.

Institutional Review Board Approval—Vaginal specimens were collected in accordance with IRB-approved protocols (IRB ID 201108155). Written informed consent was received from participants as part of the Contraceptive CHOICE project (IRB ID 201101982) prior to inclusion in this study.

RESULTS

Genomic analysis of the fully sequenced *G. vaginalis* type strain ATCC14019 revealed a putative sialidase and a nearby gene cluster encoding homologs of sialic acid catabolic enzymes (see supplemental Fig. S2). However, ATCC14019 did not produce sialidase activity in our hands when grown *in vitro* (Fig. 1*A*). Thus, to study sialidase-producing strains of *G. vaginalis*, and their ability to catabolize sialic acids from mucosal sialoglycans, we isolated new fresh clinical strains of *G. vaginalis*. Briefly, women who were enrolled in the Contraceptive CHOICE Project at Washington University provided self-collected vaginal swabs, which were transported to the Center for Women's Infectious Disease Research under anaerobic conditions. *G. vaginalis* strains were isolated as described under "Experimental Procedures," and their identities were confirmed by PCR using primers reported to be specific for genomic DNA encoding the *Gardnerella* 16 S ribosome (50). Sequencing of ~1400 base pairs of the 16 S ribosomal DNA gave 15 strains with 98.5–100% identity to the *G. vaginalis* reference strain ATCC14019 (Table 1). A single strain of *Bifidobacterium* was also isolated using this approach with 93% identity to the 16 S sequence of *G. vaginalis* (Table 1).

Kinetic analysis of 4-methylumbelliferyl-sialic acid hydrolysis using whole bacterial cultures revealed that nine of these *G. vaginalis* strains produced high levels of sialidase activity (++++, +++, or ++), one strain produced intermediate levels of sialidase activity (+), and five strains produced very low or undetectable levels of sialidase (−) when cultured anaerobically in NYC-III media (Fig. 1*A*, Table 1). These strains producing little or no sialidase activity are hereafter referred to as "sialidase-negative." Of the strains that produced sialidase, most of the enzyme activity was cell-associated and thus removed from the culture by pelleting bacteria (Fig. 1*B*). However, a fraction of the activity remained in the bacterial supernatant (Fig. 1*B*). Consistent with the quantitative analysis of enzyme activity, a chromogenic SiaX substrate analogous to the familiar X-Gal substrate revealed blue staining of bacterial colonies (supplemental Fig. S3). Staining also diffused through NYC-III agar plates surrounding *G. vaginalis* colonies, further supporting the conclusion that a portion of the *G. vaginalis* sialidase is also secreted (supplemental Fig. S3). Limited proteolysis with subtilisin released the cell-associated sialidase activity into the soluble supernatant, demonstrating that the enzyme activity is resistant to mild proteolysis and further supporting the conclusion that sialidase is localized on the surface of *G. vaginalis* (supplemental Fig. S3*C*, *arrowhead*). However, there are no canonical Gram-positive signal sequences in the amino acid sequence of the predicted sialidase according to SignalP4.1, nor are there any LP*X*TG motifs.

We measured the ability of *G. vaginalis* isolates to consume sialic acids during anaerobic growth in NYC-III media, which contains free sialic acids (~17%) as well as bound sialoglycans (~83%). Total sialic acid levels in the bacterial cultures were measured using previously described methods of fluorescent derivatization and HPLC separation (52, 53, 56). Compared with uninoculated media incubated in parallel, growth of the sialidase-positive strains resulted in progressive depletion of sialic acids from the culture media (Fig. 2, *A* and *B*). On average, sialidase-positive strains consumed about half of the total sialic acids in culture media by 8 h and about 75% of sialic acids were consumed by 24 h post-inoculation. In contrast, sialidase-negative isolates consumed little or no sialic acid (Fig. 2, *A* and *B*). Differences in growth between strains did not account for dif-

TABLE 1
***G. vaginalis* strains used in this study**

Patient	Strain name	BV status	Nugent score	Sialidase activity	No. of Identical 16 S bases/total sequenced	Identity to reference strain	GenBank accession number
NA[a]	ATCC14019	Unknown	NA	−	Reference	100.0%	
047275	JCP7275	Positive	10	−	1408/1409	99.9%	JX860309
047276	JCP7276	Intermediate	5	+++	1411/1411	100.0%	JX860310
047659	JCP7659	Positive	8	++	1397/1407	99.3%	JX860311
047672	JCP7672	Negative	3	+/−	1412/1412	100.0%	JX860312
047719	JCP7719	Positive	8	+	1385/1397	99.1%	JX860313
048017	JCP8017A	Positive	8	+++	1398/1409	99.2%	JX860314
048017	JCP8017B	Positive	8	+++	1403/1413	99.3%	JX860315
048066	JCP8066	Negative	0	++++	1382/1402	98.6%	JX860316
048070	JCP8070	Positive	8	+++	1400/1412	99.2%	JX860317
048108	JCP8108	Positive	8	−	1390/1396	99.6%	JX860318
048151	JCP8151A	Positive	10	+++	1003/1015	98.8%	JX860319
048151	JCP8151B	Positive	10	+++	1377/1394	98.8%	JX860320
048481	JCP8481A	Positive	10	−	1394/1408	99.0%	JX860321
048481	JCP8481B	Positive	10	−	1389/1410	98.5%	JX860322
048522	JCP8522	Positive	8	+++	1397/1409	99.1%	JX860323
Bifidobacterium isolate 047499	JCP7499	Positive	8	−	1332/1428	93.3%	JX860308

[a] NA, not applicable.

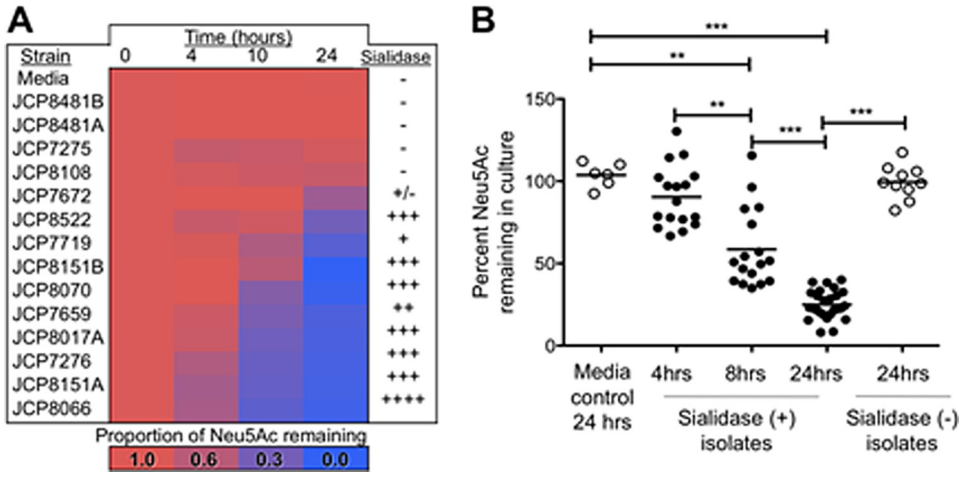

FIGURE 2. **Sialidase-positive *G. vaginalis* strains consume sialic acid from bound sialoglycans in culture media.** *G. vaginalis* isolates were cultured anaerobically in NYC-III media, and total sialic acid content of the media was measured over 24 h postinoculation. *A,* heat map of sialic acid in media shows reduction of starting Neu5Ac levels (*red*) to low levels (*blue*) by each *G. vaginalis* strain over 24 h with sialidase-positive strains. Values are means of 2–4 experiments. The time point taken at 7–12 h is reported as 10 h. *B,* pooled data of sialidase-positive strains JCP7719, JCP7659, JCP8017A, JCP7276, JCP8066, JCP8522, JCP8151B, JCP8151A, and JCP8070 at 4, 8, and 24 h compared with 24-h data from media alone control and sialidase-negative strains JCP7275, JCP8108, JCP8481A, and JCP8481B. Combined data are shown from 5 independent experiments performed on different days. Each strain was analyzed in at least 2 independent experiments. *Bars* represent median values. Statistical significance was examined using one-way analysis of variance followed by Bonferroni multiple comparisons test: **, $p < 0.01$; ***, $p < 0.001$.

ferences in sialic acid foraging under these conditions (supplemental Fig. S4). These data demonstrate that sialidase-positive *G. vaginalis* isolates are able to liberate and consume sialic acids present as bound sialoglycans in the extracellular environment.

To define the sequence of events in *G. vaginalis* sialoglycan hydrolysis and sialic acid consumption, we examined processing of the purified sialoglycoprotein substrate immunoglobulin A (IgA) by washed bacteria resuspended in acetate buffer, comparing the sialidase-positive strain JCP8151B to the sialidase-negative strain JCP8481A. Strains were mixed with human IgA and the levels of free sialic acids in the soluble extracellular compartment were measured over time. Free sialic acid in the soluble supernatant was evident within minutes, indicating extracellular hydrolysis of IgA sialic acid residues (Fig. 3*A*). The concentration of free sialic acid peaked at 40 min and progressively declined with very little free sialic acid remaining (<1 μM) after 120 min (Fig. 3*A*). These results clearly show that sialic acids on IgA are hydrolyzed outside the cell by *G. vaginalis* sialidase prior to their uptake and catabolism. Additional studies examined the ability of *G. vaginalis* isolates to consume free sialic acids when the requirement for sialidase was bypassed. Consistent with the data using IgA as a substrate, sialidase-positive strains readily consumed the provided free sialic acid. In contrast, sialidase-negative strains were unable to efficiently take up and catabolize free sialic acids (Fig. 3*B*). The lack of sialidase and catabolic activity consistently observed among sialidase-negative *G. vaginalis* isolates suggests that these strains fail to express the uptake and/or catabolic mechanisms downstream of sialidase, which are expressed in sialidase-positive strains under these conditions.

Much of the sialic acid at mucosal surfaces is found in mucins, heavily *O*-glycosylated proteins that are cell-surface associated, or secreted by specialized cells found at all mucosal sites (60–62). To investigate the utilization of mucin sialic

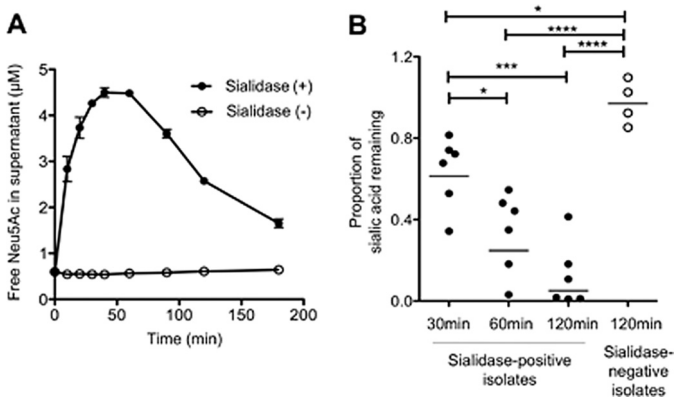

FIGURE 3. *A*, extracellular hydrolysis of human IgA sialic acids by *G. vaginalis* precedes consumption. Clinical isolates JCP8151B (sialidase-positive) and JCP8481A (sialidase-negative) were incubated with purified human serum IgA in acetate buffer and the concentration of free sialic acid in the supernatant was monitored over the course of 3 h. Analysis of 3 samples from a single *G. vaginalis* preparation are shown in this representative experiment. *B*, *G. vaginalis* clinical isolates consume free sialic acid. *G. vaginalis* strains were washed and incubated with 10 μM free Neu5Ac in acetate buffer and the sialic acid concentration was measured over a 4-h time course. Combined data from 2 independent experiments are shown. Sialidase-positive strains (JCP7719, JCP7276, JCP8066, JCP8522, JCP8151B, and JCP8070) at 30, 60, and 120 min were compared with 120-min data from sialidase-negative strains (JCP7275, JCP8108, and JCP8481A). *Bars* are geometric means. Statistical significance was examined using one-way analysis of variance followed by Bonferroni multiple comparisons test: *, $p < 0.05$; ***, $p < 0.001$; ****, $p < 0.0001$.

acids, we measured *G. vaginalis* hydrolysis and utilization of a purified preparation of mucin from bovine submaxillary glands. Surprisingly, *G. vaginalis* was much less capable of consuming sialic acid (Neu5Ac) when it was presented in the context of bovine submaxillary mucin (BSM) as compared with human IgA (Fig. 4*A*). This was not due to an inability to hydrolyze sialic acids from *O*-glycans, because free sialic acid levels displayed similar kinetics for BSM and IgA during the first 20 min after exposure to *G. vaginalis* washed whole cells (Fig. 4*B*).

We hypothesized that the reduced ability of *G. vaginalis* to consume sialic acids on BSM may have something to do with its bovine origin. BSM displays approximately equal amounts of Neu5Ac and 5-*N*-glycolylneuraminic acid (Neu5**Gc**), a hydroxylated form of 5-*N*-acetylneuraminic acid (Neu5**Ac**) that is found in all mammals except humans (63–65). Analysis of Neu5Gc during sialic acid catabolism by *G. vaginalis* revealed similar kinetics of release compared with Neu5Ac; however, the levels of free Neu5Gc remained high throughout the 4-h duration of the assay (Fig. 4*B*), demonstrating that *G. vaginalis* efficiently liberates but inefficiently consumes the nonhuman sialic acid Neu5Gc. To test the hypothesis that Neu5Gc may impair the catabolism of Neu5Ac, we performed *G. vaginalis* sialic acid utilization assays using human IgA (which contains only Neu5Ac), in the presence or absence of excess purified Neu5Gc. Consistent with the BSM experiments, these data show that free Neu5Ac (from IgA) can be liberated by *G. vaginalis* in the presence of Neu5Gc, but that free Neu5Ac is *not* consumed by *G. vaginalis* in the presence of Neu5Gc (Fig. 4, *C* and *D*). These data demonstrate that the nonhuman sialic acid Neu5Gc inhibits the catabolism of human sialoglycans by *G. vaginalis*, and that Neu5Gc interference occurs downstream of Neu5Ac hydrolysis.

To define the next steps in sialic acid utilization by *G. vaginalis* and identify pathway components affected by Neu5Gc, we measured bacterial enzyme activities involved in Neu5Ac catabolism in the presence or absence of Neu5Gc. Previous studies in Gram-negative bacteria have characterized a *N*-acetylneuraminic acid aldolase/lyase, which catalyzes the interconversion of nine-carbon backbone Neu5Ac and six-carbon backbone *N*-acetylmannosamine (ManNAc) + three-carbon pyruvate. Homologs of the sialate lyase enzyme have been shown to operate in Neu5Ac catabolism and in Neu5Ac biosynthesis in different systems (66). To investigate Neu5Ac lyase/aldolase activity in *G. vaginalis*, we first measured the ability of cell lysates to destroy Neu5Ac (lyase activity). These experiments showed that soluble cell lysates of *G. vaginalis* (after removal of any cell debris) quickly destroyed the provided Neu5Ac substrate (Fig. 5*A*). When *G. vaginalis* lysates were instead incubated with excess ManNAc and pyruvate, Neu5Ac production was observed (Fig. 5*B*). We emphasize that the amount of Neu5Ac produced in these experiments was small compared with the input of pyruvate and ManNAc. However, the data provide strong evidence that the *Actinobacterium G. vaginalis* uses a similar pathway of Neu5Ac catabolism as previously described for Gram-negative bacteria, a retro-aldol mechanism mediated by a Neu5Ac lyase/aldolase (66). The uptake of free Neu5Ac by *G. vaginalis* was markedly reduced in the presence of Neu5Gc (Fig. 5*C*), whereas investigation of sialate lyase (Fig. 5*A*) and sialidase (Fig. 5*D*) activities revealed no inhibition of activity in the presence of excess Neu5Gc.

To determine whether *G. vaginalis* sialoglycan foraging occurs within the physiologically relevant context of human vaginal fluid, we next investigated the kinetics of *G. vaginalis* sialoglycan hydrolysis and sialic acid catabolism following incubation of bacteria directly in buffered human vaginal specimens. For these experiments, we used human vaginal mucus pooled from 14 women with a normal lactobacilli-dominated microbiota as verified by Nugent score (0–3) and negative sialidase activity assay. Washed pellets of sialidase-positive and sialidase-negative *G. vaginalis* isolates were incubated with human vaginal fluid, followed by analysis of free and total sialic acid levels. Consistent with their behavior in culture, sialidase-positive *G. vaginalis* isolates hydrolyzed sialoglycans in human vaginal mucus, resulting in a pool of free sialic acids that was evident within minutes and destroyed over time (Fig. 6*A*). Depletion of the free sialic acid pool by *G. vaginalis* was mirrored by reductions in the total sialic acid, with virtually no intact mucus sialoglycan remaining after 120 min (Fig. 6*B*). In contrast, mucus sialoglycans remained intact in the presence of sialidase-negative *G. vaginalis* (Fig. 6, *A* and *B*). These data suggest that the degradation and catabolism of human vaginal sialoglycans proceeds through the same pathway as observed for the purified human sialoglycan substrate IgA (Figs. 3*A* and 4).

To test the potential for sialic acid catabolism by *G. vaginalis* in the context of the mouse vagina, isolates were first incubated with mucus derived from pooled mouse vaginal washes. After a 4-h incubation, sialidase-positive isolates reduced the total sialic acid pool by about 75% (Fig. 6*C*). Sialidase-negative strains had a negligible impact on total sialic acid levels in mouse vaginal mucus. Next, we tested whether *G. vaginalis* is

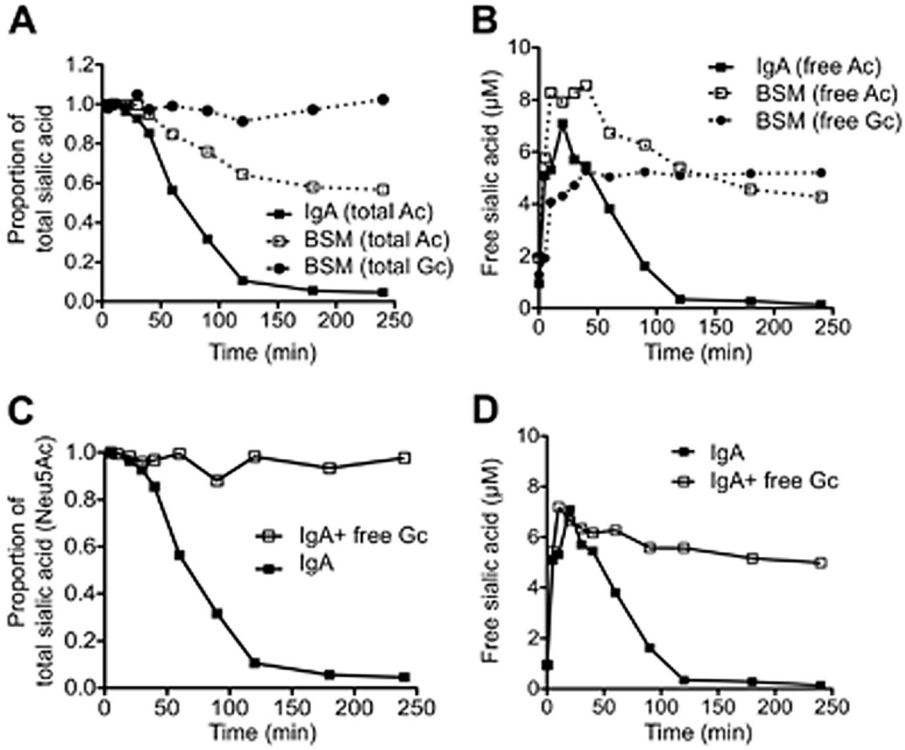

FIGURE 4. **The rate of *G. vaginalis* sialic acid (Neu5Ac) consumption from sialoglycans is substrate-dependent and inhibited by *N*-glycolylneuraminic acid (Neu5Gc).** Washed *G. vaginalis* JCP8151B was incubated with 0.2 mg/ml of human IgA or 0.3 mg/ml of BSM, followed by kinetic measurements of total (*A*) and free (*B*) sialic acids. The total starting concentration of Neu5Ac in each experiment was ~15 μM. *Ac* and *Gc* refer to quantitation of Neu5Ac or Neu5Gc, respectively, in reactions with BSM. *C* and *D,* added Neu5Gc inhibits consumption but not release Neu5Ac from IgA by *G. vaginalis*. Washed *G. vaginalis* JCP8151B was incubated with human IgA with or without 150 μM Neu5Gc ("+ free Gc") and levels of total (*C*) and free (*D*) Neu5Ac were determined. These data are representative of 3 or more independent experiments.

sufficient to degrade, catabolize, and deplete sialic acids from the vaginal mucosa *in vivo*. Sialidase-positive *G. vaginalis* strain JCP8151B was inoculated into the vaginas of C57/BL6 mice, followed by measurement of free and total sialic acids in vaginal washes as described under "Experimental Procedures." Control groups of mice received an equal volume of vehicle alone and were washed in parallel with *G. vaginalis*-infected mice at 24 and 72 h post-infection. At both time points, pinpoint colonies of *G. vaginalis* were re-isolated from murine vaginal washes by plating on semiselective media, cultured under anaerobic conditions, and confirmed by PCR. Resident bacteria also grew on "Gardnerella semiselective" agar, resistant to colistin, nalidixic acid, trimethoprim sulfamethoxazole, and other antibiotics, obscuring an absolute estimation of *G. vaginalis* vaginal titers. We note that previously published models of *G. vaginalis* vaginal infection did not address this potentially confounding factor (67, 68).

Biochemical analyses of vaginal washes at 24-h postinfection revealed significantly higher levels of *free* Neu5Ac in the *G. vaginalis*-infected mice compared with mock-infected animals, indicating that the active breakdown of vaginal mucus sialoglycans by *G. vaginalis* occurs *in vivo* (Fig. 7*A*). By the 72-h time point, *G. vaginalis*-infected mice exhibited significantly lower levels of bound Neu5Ac (Fig. 7*B*), in vaginal washes compared with mock-infected animals. Together these data show that *G. vaginalis* participates in the hydrolysis and foraging of vaginal mucus sialoglycans *in vivo*, depleting more than half of the bound Neu5Ac content in vaginal washes (mean 19.61 μM, confidence interval 13.96–25.25) compared with mock-infected animals (mean 41.38 μM, 95% confidence interval 25.73–57.03).

To investigate the evidence that sialic acid hydrolysis, foraging, and depletion occurs in the clinical setting of BV, we performed biochemical analyses of eluted vaginal fluids from women with bacterial vaginosis (Nugent score 7–10) compared with women with a normal lactobacilli-dominated microbiota (Nugent score 0–3). Measurements of free and total sialic acid levels in these specimens revealed that women with BV had >3-fold *higher* free sialic acid levels (mean 20.7 μM, 95% confidence interval 11.3–30.0 μM) compared with normal controls (mean 5.8 μM, 95% confidence interval 3.7–7.8 μM) (Fig. 8*A*). These data are consistent with a steady release of free sialic acids in vaginal mucus due to bacterial sialidase activity associated with BV. In contrast, measurements of total sialic acids in vaginal fluids revealed that women with BV had >3-fold *lower* levels of total sialic acid (mean 48 μM, 95% confidence interval 32.7–63.4 μM) compared with women with a normal lactobacilli-dominated microbiota (mean 158 μM, 95% confidence interval 104.5–212.1 μM) (Fig. 8*B*). These data strongly suggest that the hydrolysis, catabolism, and depletion of vaginal mucus sialic acids observed in our experimental models are also occurring in women with BV.

DISCUSSION

"Dysbiosis" refers to the idea of an imbalance between "beneficial" and "harmful bacteria" at mucosal surfaces, a concept that has increasing support in gut pathologies such as inflam-

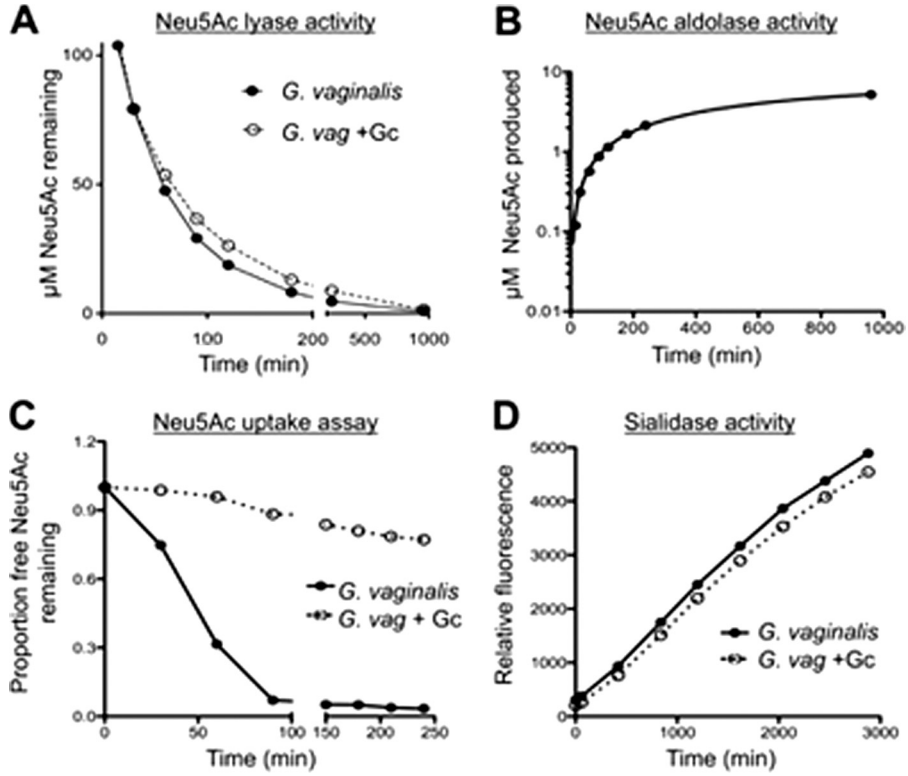

FIGURE 5. **Excess Neu5Gc interferes with Neu5Ac transport, but not sialidase or lyase activities.** Cell lysates of *G. vaginalis* JCP8151B were prepared by sonication and centrifugation of cell debris, and then lysates were mixed with enzyme substrates. *A,* Neu5Ac (100 μM) was consumed within hours, consistent with sialate lyase activity. *B,* ManNAc and pyruvate (1 mM each) allow cell lysates to synthesize micromolar concentrations of Neu5Ac in the reverse (aldolase) reaction. *C,* uptake and catabolism of Neu5Ac by live *G. vaginalis* was performed after washing cells as described under "Experimental Procedures." *D, G. vaginalis* sialidase activity was measured using the fluorescent 4-methylumbelliferyl-Sia substrate. The non-human sialic acid Neu5Gc (100 μM) "+ Gc," slows the uptake of free Neu5Ac by *G. vaginalis*, but does not inhibit sialidase activity, or Neu5Ac lyase activity. These data are representative of three or more independent experiments.

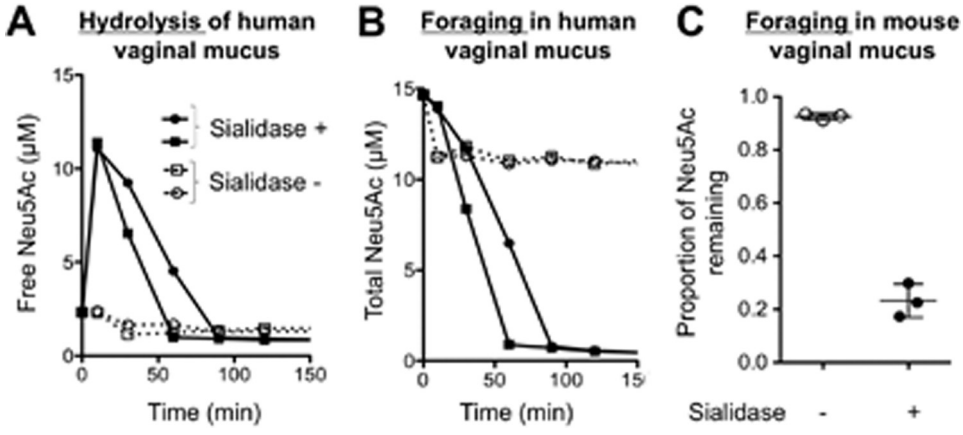

FIGURE 6. ***G. vaginalis* liberates and consumes sialic acids from human and mouse vaginal mucus *in vitro*.** *A* and *B, G. vaginalis* sialidase-positive strains (JCP8151B, JCP8017A) and sialidase-negative strain (JCP8481A, ATCC14019) were incubated *in vitro* with vaginal mucus eluted from human vaginal swabs (pool of 14 BV-negative, sialidase-negative samples) followed by measurement of free (*A*) and total (*B*) sialic acids acid by HPLC as described under "Experimental Procedures." Representative time courses are shown from three independent experiments. *C, G. vaginalis* sialidase-positive strain JCP8151B and sialidase-negative strain JCP8108 were incubated for 4 h *in vitro* with vaginal mucus from mouse vaginal washes (pooled from uninfected C57/Bl6 mice), followed by measurement of total Neu5Ac by HPLC. Data points are shown from 3 independent experiments performed on different days. Mean values with S.D. are shown.

matory bowel disease (69–71) and vaginal conditions such as bacterial vaginosis.

Here we provide the first report of a BV-associated bacterium, *G. vaginalis*, which actively participates in the degradation of protective vaginal mucus barriers. The data presented here also demonstrate Nugent-defined BV as the first example, to our knowledge, of a microbial imbalance or dysbiosis in humans in which protective mucus sialoglycan barriers are measurably degraded and depleted from affected mucosal specimens compared with normal controls.

We show that *G. vaginalis* sialic acid foraging was sufficient to cause mucosal sialic acid depletion in a mouse vaginal infection model. The degree of sialic acid depletion observed in clinical specimens in the current study is consistent with our pre-

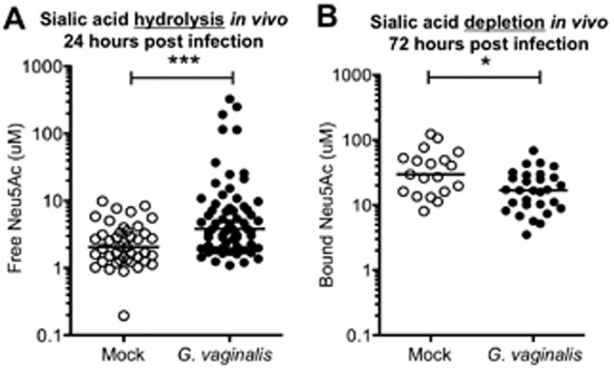

FIGURE 7. ***G. vaginalis* liberates and depletes sialic acids from vaginal mucus barriers *in vivo*.** 1×10^6 *G. vaginalis* isolate JCP8151B was inoculated vaginally into C57Bl6 mice. Vaginal washes were collected at 24 and 72 h postinfection using 50 μl of phosphate-buffered saline. Free (*A*) and bound (*B*) Neu5Ac was measured by HPLC as previously described at 24 and 72 h post-*G. vaginalis* infection, respectively. Combined data of 2–3 independent experiments are shown ($n = 10$ animals per group). *Bars* represent median values. Note log scales. *, $p < 0.01$; ***, $p < 0.0001$.

vious work on BV specimens showing that sialic acids provided *exogenously* in various contexts (*e.g.* α2–3- and α2–6-linked sialic acids found on both *O*-linked and *N*-linked sialoglycoproteins) are effectively cleaved by sialidases present in BV vaginal fluids, but not by healthy controls (52). The data further demonstrate that sialidase-positive *G. vaginalis* strains isolated from women with BV are capable of hydrolyzing vaginal sialoglycans, including IgA and mucin, and *G. vaginalis* was sufficient to result in depletion of vaginal sialic acids within 72 h in a murine model. *G. vaginalis* secretion and surface presentation of sialidase enzyme allowed for the extracellular hydrolysis of bound Neu5Ac-containing vaginal mucus sialoglycans, followed by transport of Neu5Ac into the intracellular compartment (see model, Fig. 9). Following uptake, catabolism of sialic acid proceeded without accumulation of intracellular Neu5Ac (intracellular Neu5Ac was detectable in low concentrations in our hands) through a mechanism involving a sialic acid aldolase/lyase reaction. Although total levels of sialic acid were lower in BV vaginal fluids, the levels of free sialic acid in BV clinical specimens were often elevated, consistent with the "release and capture" biochemical pathway demonstrated *in vitro* and *in vivo* with *G. vaginalis*.

The machinery for bacterial sialic acid catabolism has been described in several other systems, mostly in aerobic Gram-negative *Proteobacteria* (72–77). Sialic acid catabolism has also been recently demonstrated to support growth of the Gram-positive bacterium Group B *Streptococcus* in a vaginal model of infection (78). Interestingly, this bacterium does not encode a putative sialidase or express sialidase activity when grown in culture, suggesting that it may have a competitive advantage in the BV vaginal environment where we show (Fig. 8) free sialic acid levels are much higher than in a lactobacilli-dominated vaginal environment.

Foraging on Sialic Acid in the Vagina—Metabolic machinery for sialic acid hydrolysis and utilization has been implicated in gastrointestinal, respiratory, and periodontal pathogenesis of several bacterial species, including *Vibrio cholerae* (79), *Streptococcus pneumoniae* (80), and *Tannerella forsythia* (81, 82). The normal commensal microbiota of the adult oral gastrointestinal mucosa such as *Streptococcus oralis* and *Bacteroides thetaiotaomicron* have also been shown to hydrolyze sialic acids and utilize host mucus under certain conditions (83–86). Other studies suggest that in the infant gut, the beneficial commensal *B. longum* subspecies infantis utilizes a sialidase to hydrolyze sialic acid-containing oligosaccharide "prebiotics" secreted by the maternal mammary gland (87, 88). However, to our knowledge, this is the first description of sialoglycan foraging by a sialidase-expressing *vaginal-adapted* bacterium. In contrast to oral and gastrointestinal environments, where sialidase activity is ubiquitous, BV presents a unique opportunity for the study of disease-associated sialidase activity. Detectable sialidase activity in vaginal fluids occurs almost universally in BV, and rarely if ever in normal controls with vaginal microbiota dominated by lactobacilli (14, 35–38).

Sialidase Status of G. vaginalis Isolates—Other papers have shown that some *G. vaginalis* strains are sialidase-positive, whereas others are negative (34, 89, 90), a finding we confirm among our *G. vaginalis* isolates (Fig. 1, Table 1). One recent study that investigated sialidase activity and the presence or absence of a gene encoding a putative sialidase open reading frame (by PCR) concluded that sialidase-negative strains lack the sialidase gene (89). Another similar study concluded that phenotypically sialidase-negative strains can encode a predicted sialidase, whereas failing to express the active enzyme in culture (90). Among our *G. vaginalis* strains, we confirmed the presence of a ~3-kilobase open reading frame encoding a putative sialidase homolog, including phenotypically sialidase-negative JCP8108 and JCP7275 (supplemental Fig. S5). This finding is consistent with our observation that strain ATCC14019 encodes the genes for sialidase and sialic acid catabolism (supplemental Fig. S2), whereas being phenotypically sialidase-negative. It is possible that sialidase and other components of the pathway may be differentially regulated among sialidase-positive and sialidase-negative strains of *G. vaginalis*. Further studies are required to define and understand the behaviors of *G. vaginalis* isolates under different environmental conditions.

Neu5Gc As an Inhibitor of G. vaginalis Neu5Ac Uptake—Two major forms of sialic acids differing by a single oxygen atom are found in mammals, Neu5**Ac** and Neu5**Gc**. The hydroxylated acetylneuraminic acid Neu5Gc is found alongside Neu5Ac in all mammals examined, except humans, due to a genetic event that inactivated the enzyme responsible for synthesis of Neu5Gc from Neu5Ac. *G. vaginalis* sialidase does not appear to have strong preferences between Neu5Ac and Neu5Gc as substrates. However, the uptake and catabolism of sialic acids was substrate dependent and occurred much more slowly and incompletely in the context of significant amounts of Neu5Gc. The presence of Neu5Gc during Neu5Ac uptake lead to higher concentrations of free extracellular Neu5Ac, showing that Neu5Ac is indeed released, but taken up more slowly. Addition of excess Neu5Gc did not inhibit the liberation of Neu5Ac by *G. vaginalis* sialidase or the catabolism of Neu5Ac by cell lysates, showing that Neu5Gc acts downstream of sialic acid hydrolysis, yet upstream of the catabolic machinery, beginning with the intracellular lyase enzyme, together suggesting that Neu5Gc inhibits Neu5Ac transport into *G. vaginalis*. These results are similar to a previous observation

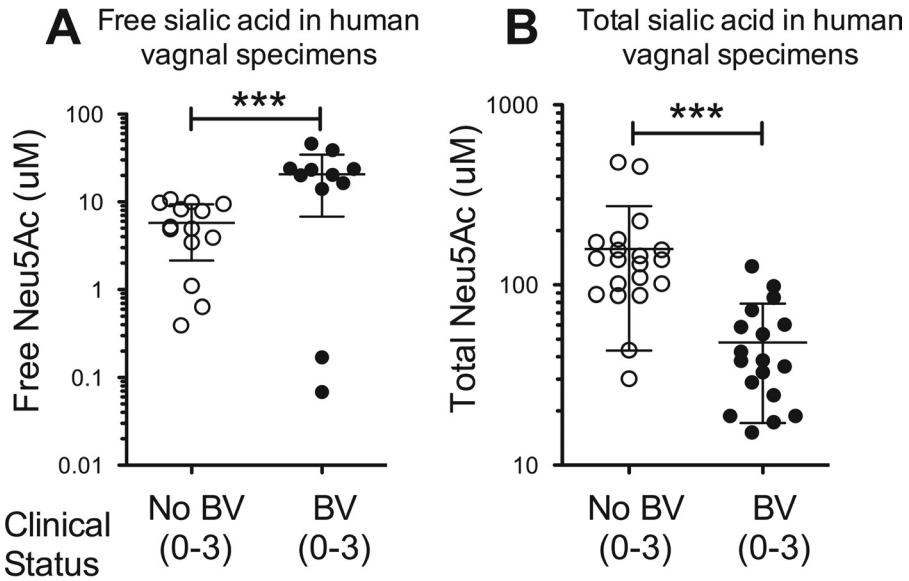

FIGURE 8. **New biochemical features of human BV: sialic acid hydrolysis and depletion.** Vaginal specimens were assigned a clinical status of *BV* or *No BV* based on the method of Nugent scoring of Gram-stained slides (score ranges shown in *parentheses*). Vaginal swabs were eluted and subjected to biochemical analysis of free (*A*) and total (*B*) sialic acid (*Neu5Ac*) levels as described under "Experimental Procedures." A total of 63 individuals were studied. Median values with S.D. are shown. Note log scales. Statistical significance was evaluated using the nonparametric Mann-Whitney *U* test. ***, $p < 0.001$.

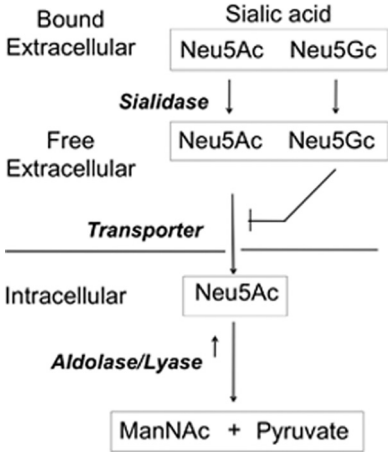

FIGURE 9. **Release and capture model of *G. vaginalis* sialic acid utilization.** 1) The secretion and extracellular localization of sialidase enzyme allows for the release of sialic acids from mucosal sialoglycan substrates outside the cell; 2) a transport system pumps the liberated free Neu5Ac into the cell, a process that can be inhibited by Neu5Gc; and 3) intracellular catabolism is initiated by Neu5Ac lyase/aldolase.

that Neu5Gc inhibits the uptake of Neu5Ac in a distantly related Gram-positive bacterium *S. oralis* (91). Addition of exogenous glucose dramatically increased the rate of Neu5Ac uptake and catabolism (supplemental Fig. S6), suggesting that transport of Neu5Ac in *G. vaginalis* may be an active process. We also note that at the completion of our uptake assays, the remaining extracellular Neu5Ac levels are low (around 1 μM). Taken together, these data strongly suggest a high-affinity transport system in *G. vaginalis* with a preference for Neu5Ac.

There are over 50 structurally distinct sialic acids reported and it is possible that other structural variations of sialic acid may influence whether *G. vaginalis* sialidase or uptake machinery are effective on these molecules. In particular, *O*-acetylation of the 3-carbon sialic acid side chain has been known to influence that biological properties of sialic acids and to be resistant to cleavage by many sialidases (57, 92, 93). In fact, our studies strongly suggest that *O*-acetylation protects sialic acids from cleavage by *G. vaginalis* sialidase (data not shown).

Foraging of Sialoglycans, A Normal or Pathologic Process?—The depletion of mucosal sialic acid in BV has a number of interesting biological, immunological, and clinical implications. Mucus is widely held as a first-line of defense that protects mucosal epithelial surfaces from direct contact with bacteria. Mucus contains a high concentration of glycoproteins, including immunoglobulins and mucins, which have defined roles in the exclusion of potential pathogens from mucosal surfaces. Vaginal sialidase activity has been correlated with adverse obstetric and gynecological outcomes in several clinical studies, suggesting that sialic acid foraging by BV-associated bacteria may lead to increased risk of reproductive host pathology. In most cases that have been studied so far, bacterial sialidases and/or sialic acid catabolic enzymes do contribute to virulence and/or colonization by bacterial pathogens. However, as described above, there are several examples of commensal bacteria that seem to strike a balance with the host, foraging on mucus sialic acid residues without damage to host tissues. Further studies should examine whether and how mucosal depletion of sialic acids may contribute to the general risk of secondary infection associated with BV.

Sialoglycan Depletion as a Community Activity?—We emphasize that other BV-associated bacteria or combinations of BV-associated bacteria may also be capable of hydrolysis and depletion of mucosal sialic acids. In fact, nearly all vaginal isolates from the phylum *Bacteroidetes* can produce sialidase enzyme, including isolates from the BV-associated genus *Prevotella* (32, 34, 94). The observation that women with BV have significantly lower levels of vaginal mucus sialic acids is likely the result of a milieu of enzymes produced by distinct communities of bacteria in individual women. It may be of interest in future studies to examine potential relationships between community composi-

tion, sialidase levels, and the extent of sialic acid depletion. Sialidase action on mucus sialoglycans may also promote further mucus degradation by revealing underlying substrates that can be cleaved by other glycosidases produced by vaginal bacteria. Evidence of enhanced glycan degradation in bacterial dysbiosis has been reported in human vaginal fluids (52) and fecal specimens (95). These studies provide a template for integrating knowledge gained from clinical correlations and microbiome studies together with experimental models aimed at understanding the potential contributions of different community members to the degradation of protective mucus barriers in BV.

Finally, recent studies have demonstrated correlations between particular BV-associated bacteria and certain clinical features of BV (26). However, causal relationships between individual bacterial species and measurable clinical phenotypes of BV have been elusive. Here we demonstrate the consequence of an accepted diagnostic feature of BV, vaginal sialidase activity, on mucosal sialoglycans of women with this condition. The data presented here show that sialic acid residues are both liberated and depleted in BV compared with normal controls, and demonstrate that *G. vaginalis* is sufficient to elicit this phenotype in an animal model, and moreover, provide a detailed understanding of *G. vaginalis* sialic acid foraging at the biochemical level. These findings demonstrate mucosal sialic acid depletion as a new clinical feature of BV and provide the first example of a BV-associated bacterium, *G. vaginalis*, which plays an active role in the degradation of protective vaginal mucus barriers.

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